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Ultrasound images were acquired from 71 patients with 80 suspicious masses. Images of 68 masses and tissue samples from 31 patients were analyzed for microvascularity. The vascularity of each mass was characterized using six different measurements representing red blood cell density, mean flow velocity, area of perfusion, blood volume, and blood flow. The measurements were made over the entire mass and also regionally. By every measure used the malignant masses were found to be more vascular than the benign lesions. The use of vascularity with grayscale ultrasound imaging increased the confidence in diagnosis. However, a notable overlap between the values of vascularity was observed for the benign and malignant groups on an individual case basis. The regional measurements showed center of the malignant masses to be less vascular than the periphery, whereas the benign masses had fairly uniform distribution. We believe that the gradient in vascularity is a measure of aggressiveness of the tumor growth, and provides information on the supply and demand requirements of tumor. The project has been successfully completed and the quantitative scheme of measuring vascularity that this research has generated should allow differential diagnosis with higher confidence, and permit an objective evaluation of tumor response to treatments in patients.

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1. Sehgal, Chandra
2. Arger, Peter
3. Rowling, Susan
4. Conant, Emily
5. Sullivan, Daniel
6. Reynolds, Carol

5. Introduction

Many studies have shown a link between tumor growth patterns and the distribution of microvessels in tissues. The growth of a solid tumor beyond a size of 2 mm (about 10^6 cells) requires a continual neovascularization to supply oxygen and nutrients to the cells [1,2]. This increase in the number of new capillaries also implies a higher probability for the tumor cells to enter blood circulation, thus creating a greater likelihood of metastasis [3]. There is convincing evidence that microvessel density (# of vessels per unit area of the tissue) is predictive of metastatic disease in breast carcinoma [2,4-6], and an independent prognostic indicator of cancer reoccurrence in patients with node-negative carcinoma [7]. Thus, in the evaluation of breast cancer the role of the growth and the development of microvessels (angiogenesis) must be considered. The difficulty in including these factors in a routine clinical examination is that the current methods of assessing angiogenesis can only be used on surgical samples. A technique that can non-invasively image microvessels and provide an estimate of its density is highly desirable. A successful development of such a technique could have a beneficial impact on the evaluation and management of patients with breast cancer. This would not only help in detecting patients with high risk, but may assist in differential diagnosis, and also aid in monitoring the changing status of the disease as a result of therapeutic interventions.

We believe ultrasound imaging, which allows direct visualization of blood vessels, is a strong candidate to achieve this goal. The purpose of this study is to determine the potential of Doppler ultrasound imaging in evaluating breast vascularity in patients with suspicious masses.

6. Progress Report Body

6.1. Methods and Results

The proposed work was performed by a team of researchers at the University of Pennsylvania. Dr. Sehgal PhD coordinated the overall study, organized the imaging protocol, recorded and digitized B-mode and Doppler images, made measurements and analyzed the data; Dr. Conant MD, selected the patients suitable for the proposed study; Drs. Arger MD and Rowling MD performed color and power Doppler imaging using state of the art imaging equipment (ATL 3000, ATL, Bothell, WA); and Drs. Reynolds and Lawton performed histologic classification of tissues and microvessel density analysis.

Patient Selection

This study was performed on patients who chose to undergo breast surgery at the Hospital of the University of Pennsylvania. Patients with a suspicious breast mass, either benign or malignant with no other previous primary cancer were selected for the study. Ultrasound imaging was performed immediately after mammography and before any diagnostic procedure (fine needle aspiration, core biopsy, or excisional bx) to avoid any disturbance of blood flow. For each patient the following clinico-pathologic characteristics were recorded: age, histologic type, tumor size and grade.

Ultrasound Imaging

B-mode and Doppler imaging (including color flow and power) were performed using an ATL3000 (ATL, Bothell, WA) scanner. The largest cross-sections of the lesion were identified in saggital and transverse planes. The diameter, D_1, and the diameters D_2

and D_3 were measured in the transverse and the saggital planes respectively. These measurements were in turn used to compute the volume V of the mass by the formula: $V=0.5*D_1*D_2*D_3$.

The color Doppler images were recorded on a video tape at the lowest possible wall filter without causing aliasing of the images. The Doppler gain was kept constant for all the studies, except in three patients. The images were acquired in theorthogonal and saggital planes of each patient. Following this, power Doppler images were recorded in the planes close to those chosen for color-Doppler imaging. All the images were recorded on a video tape. The imaging parameters used are summarized in Table 1.

Table 1: Ultrasound scanner settings used for B-mode and color and power Doppler imaging.

	Color map	Wall filter	PRF	Gain	Velocity scale
CD	1 and 4	Medium	1000	78	6.4
PD	1	Medium	1000	80	
Grey scale	Map	Dyn. Range	Persistence	Frame Rate	
	6	55 dB	medium		

Computer Image Analyses

Color and power Doppler images were digitized from the video tapes at 24 bit resolution. Five images were obtained in different planes covering the entire volume of the detected mass. The images were analyzed by the software developed by us at the University of Pennsylvania. First, the user outlines the lesion on an image. The computer determines the area of the lesion and draws one region in the center of the lesion with one forth area (half the diameter of the lesion), and a second region that surrounds the lesion with an area equal to that of the lesion. The three regions of interest are shown in Figure 1

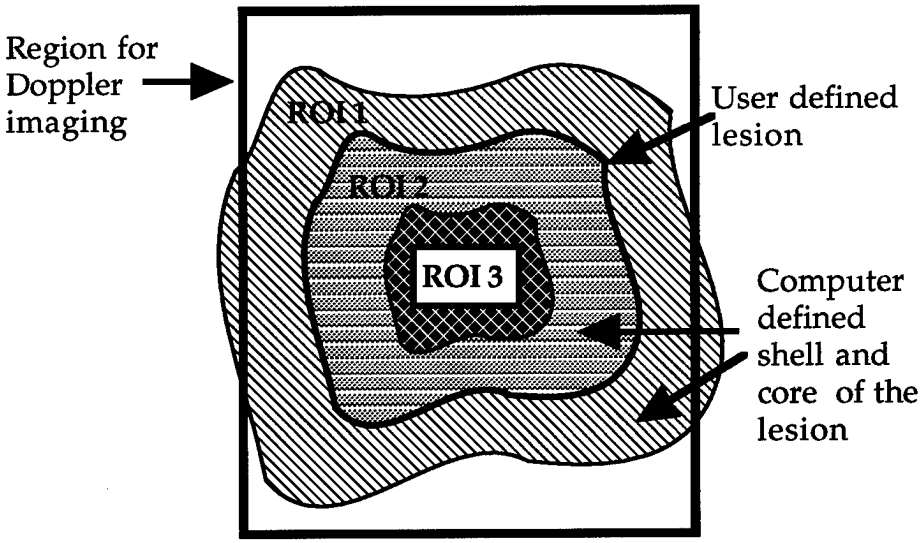


Figure 1: The three regions of interest used for making measurements. User defines the lesion on image shown as ROI 1. Following this the computer draws a shell around the image with area equal to that of the lesion ROI 2, and a core in the center with one fourth the area (half the diameter), ROI 3. The rectangle around the lesion defines the region of color and power Doppler imaging. If the lesion extends beyond this rectangle, as shown in the this figure, the computer program automatically excludes the area outside the rectangle.

On each of the regions, referred from here on as core/center C, peripheral P, and outside shell regions O, computer analyses of color in the images were made. This involved reading the color palette on the images, assigning a value of 0 to the "lowest" and a value of 100 to the "highest" color in the palette bar. With this information the computer program constructs a "look up" table from the hue, saturation, and brightness values of the colors present in the palette bar. Using the look-up table, the computer identifies the colored pixels within the region outlined by the user. It counts the number of colored pixels (n) and the number of pixels identified as the tumor (N). It also measures the color level of each pixel determined by the hue, saturation, and brightness values. Using these measurements the flow indices viz., vascular density (VD), mean color level (MCL), and color-weighted fractional area (CWFA), were calculated for each color and power Doppler image.

If i represents a color pixel, and C_i its color level, then the vascular density (VD) (also referred to as fractional area of perfusion) and mean color level (MCL) were determined by the formulas

$$VD = \frac{100 * \sum_{i}^{n} i}{N} = \text{percentage area of the lesion occupied by blood vessels, and}$$

$$MCL = \frac{\sum_{i}^{n} C_i i}{Gn},$$

respectively, where G represents the scaling factor, with values ranging from 0 to 1, for the color gain used during imaging. As described above this level was kept fixed for all our studies except in three cases.

The product of the two parameters was used to calculate color-weighted fractional area (CWFA),

$$CWFA = MCL * VD$$

The physical meaning of the measurement VD is straight forward as it represents the relative area of perfusion. The meaning of the term mean color level MCL, varies depending on whether it is derived from color or from the power Doppler images. The MCL measurements from color Doppler images is a measure of mean local blood flow velocity. On the other hand, the MCL measurements derived from power Doppler images represents the number of red blood cells moving above a threshold velocity. If one assumes local hematocrit in these small blood vessels to be equal to systemic hematocrit, MCL can be regarded to be related to blood volume (or, more appropriately related to the log of blood volume because the signals are often log compressed) moving above a threshold velocity.

The relationship between MCL and physiological parameters (mean flow velocity and blood volume) should be viewed to be semi-quantitative because of several variables; the angle between a blood vessel and the direction of ultrasound, the choice of scale maximum, filters and color write priority threshold, and, the interpolation, averaging and other image processing algorithms used internally within a scanner to display images for optimal viewing. However, if the imaging parameters are kept constant throughout the study, the influence of these variables can be significantly reduced and under these circumstances the data provides a meaningful comparison of the flow characteristics through the benign and the malignant lesions. Several studies in the last two years have demonstrated that this can indeed be achieved by careful control of image parameters [13-15].

Color weighted fractional area (CWFA), combines the MCL and VD information. For the case of color Doppler, CWFA (flow mean velocity X area of perfusion) corresponds to the blood flow through the region of interest. Similarly the color-weighed fractional area for power Doppler images, product of MCL and VD, is a measure of "moving" blood volume within the tissue.

Tumor Collection and Histology

The surgical breast specimens were fixed in 10% formalin, embedded in paraffin, and sectioned at 5 mm thickness in accordance with standard methods and stained with hematoxylin-eosin (H&E). These specimens were examined and representative sections (maximum of three) of the tumor were selected for quantitation of microvessel density (see microvessel staining, grading and counting). The WHO classification by Azzopardi [11] was used to identify the histologic type of the breast tumor. The histologic grading was performed on H&E sections according to the Elston modification of the Bloom and Richardson criteria [12]. This grading system combines three morphologic features of infiltrating breast carcinomas into a final grade [extent of glandular differentiation, nuclear grade and highest mitotic count in a representative area of 10 high-powered fields (40X objective, 10X ocular)].

Microvessel Staining, Grading and Counting

As mentioned in Tumor Collection and Histology, the specimens were examined with H&E stained sections to select three representative areas of the primary tumor for quantitation of microvessel density. Each area selected was sectioned at 5 mm and the microvessels were highlighted by staining endothelial cells for factor VIII-related antigen and CD31 using a standard immunoperoxidase staining technique. Microvessel density was determined in the areas of tumor containing the highest numbers of capillaries and small venules (microvessels) per most intense neovascularization [2]. Briefly, these neovascularized "hot spots" were detected by scanning the primary tumor sections at low power (4X and 10X) and identifying the area of most intense neovascularization. Individual microvessel counts were then be made on a 200X field (20X objective, 10X ocular) within the tumor "hot spot". Microvessels considered were individual endothelial cells or endothelial cell clusters that stained positive for factor VIII-related antigen and/or CD31. Results were expressed as the highest number of microvessels identified within any single 200X field and the average count of microvessels in the three sections were recorded for each antibody. The microvessel density and average number of microvessels were correlated with the Doppler imaging results. The results from Doppler imaging were not known to the pathologist assessing microvessel density.

Sampling

Under ideal conditions the imaging and histology measurements must be made at exactly the same site of the tumor. In a real situation it is not feasible to match the two sampling sites. This can lead to differences in measurements because the arterial and capillary networks are complex, asymmetric, and unevenly spaced over the cancer volume [10]. To minimize the sampling variations the MVD and imaging measurements were made in several planes and the mean of these values were used to establish a correlative relationship.

6.2. Results

General Characteristics

To date, we have imaged 72 patients. The mean age of the patients is 53.9 $\pm$ 14.5 years and ranges between 25.6 -79 years. The age distribution is shown in Figure 2.

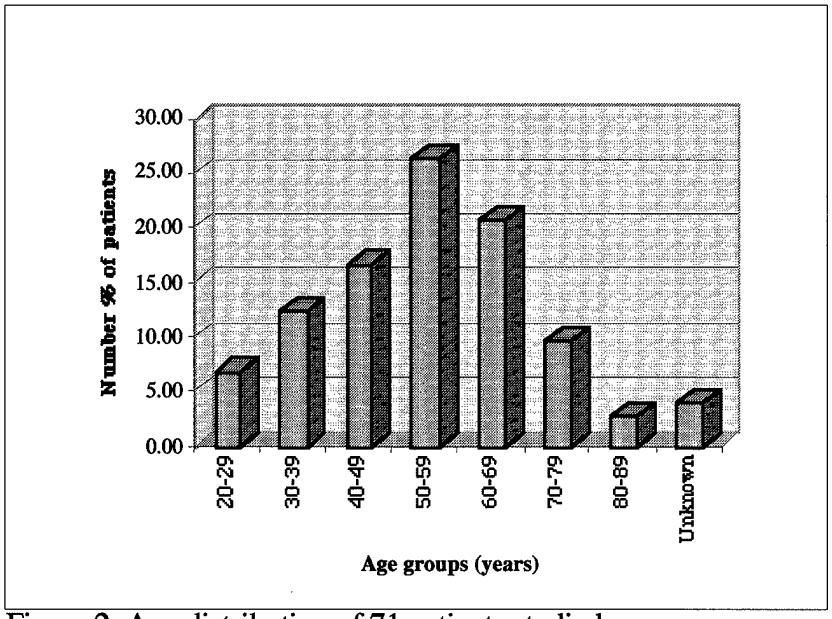
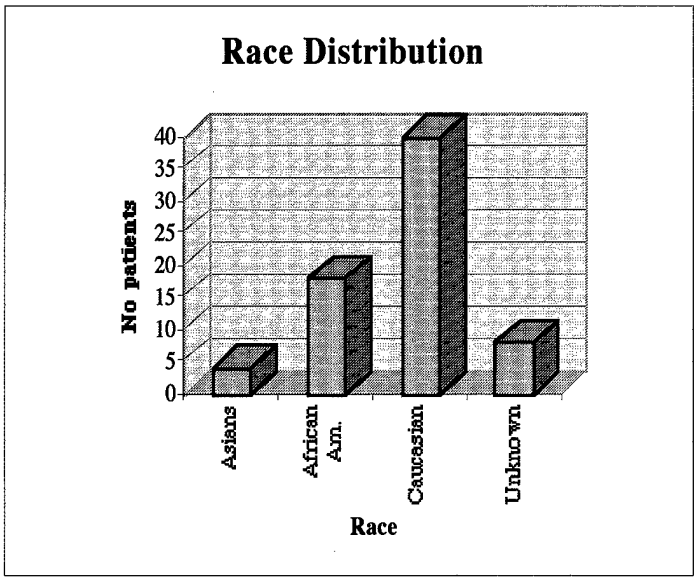


Figure 2: Age distribution of 71 patients studied.

Of the 71 patients 40 are Caucasians, 18 are African-American, 4 are Asian and 9 are unknown. This distribution is shown graphically in Figure 3. Tissue samples were collected from 33 patients.

Nine patients had two masses. Of the 80 masses, images of 68 were analyzed. The remaining 14 were not analyzed because the patients did not have a biopsy (6), the quality of images were poor (3), suspicious masses were cysts (2) or a problematic lymph node (1), and in 2 cases the masses could not be seen using ultrasound.

Figure 3: Race distribution of the patients studied



Thirty six masses were characterized as malignant including infiltrating ductal carcinomas (9), in situ and infiltrating ductal carcinomas (6), lobular carcinomas in situ (3) and mixed ductal/lobular carcinomas (4), not other wise specified (3), invasive carcinomas (11) (more details on these masses are not available at this writing). Thirteen masses were characterized as benign including 12 fibroadenomas, and 1 benign phyllodes. Eleven were grouped as atypical for this analysis. They included adenosis with lactational changes (2), benign breast tissue with fibrocystic changes (1), fibrous wall with cyst rupture (2), reactive lymph node (1), lymphoma (2), focal ADH (2), and fibrocystic changes (1). Histologies of eight are not yet available and their corresponding image analyses have not been included in the current analysis. The mean age of patients with benign and malignant lesions was 44.5 ± 14 and 59.4 ± 14.

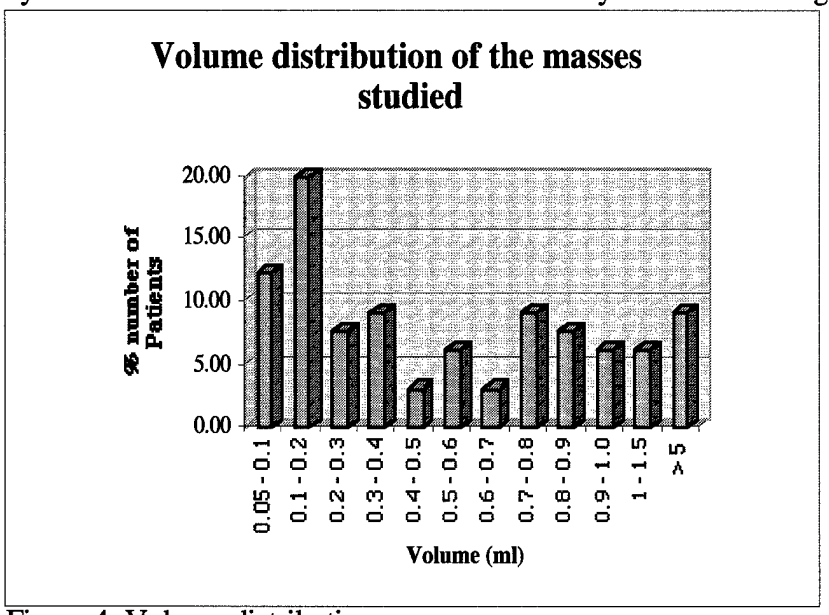


Figure 4: Volume distribution

The size of the lesions ranged from 0.05 - 19 ml (average ± std, 1.13± 2.72 ml). The largest number of masses were between the 0.05 to 0.2 ml range (Figure 4). Over the rest of the volume range the masses were fairly uniformly distributed. In terms of mean diameter, most of the masses range between 0.5-1.3 cm (Figure 5).

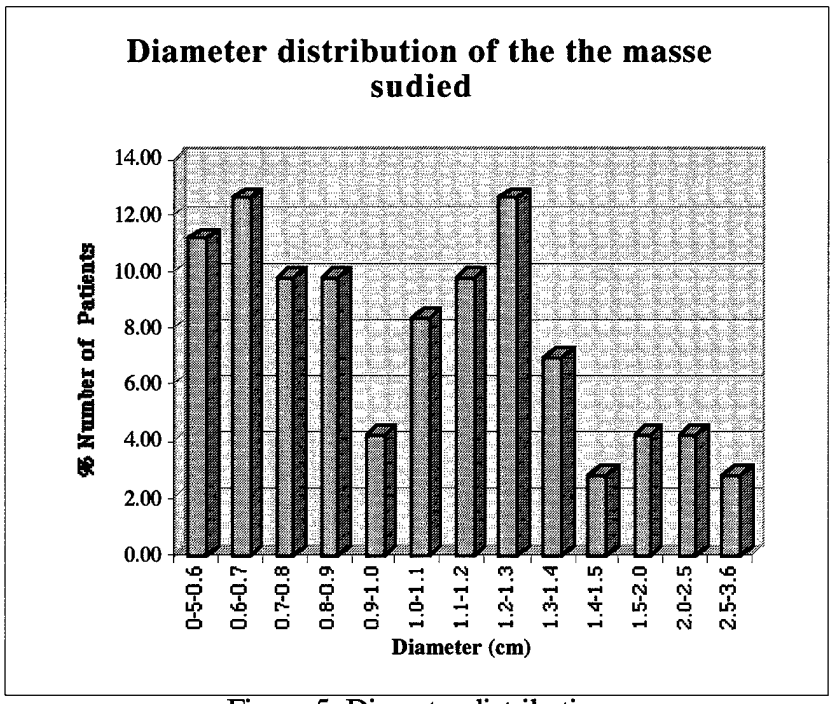


Figure 5: Diameter distribution

Imaging Characteristics

Blood vessels were detected with color Doppler (CD) imaging in all cases and in 59/60 (98%) cases with power Doppler (PD). Generally, avascular masses are considered benign whereas masses with visible vessels are considered malignant. In our studies blood flow was seen in nearly all benign cases although in most cases the vascularity was low. In malignant masses relatively stronger flow was noticed. However, there were cases where the malignant masses had moderate to low vascularity. As a consequence of this overlap there was some confusion as to the diagnosis of malignancy only on the basis of degree or visibility of blood vessels. The pattern of vascularization was often useful. In the benign masses the blood vessels were relatively uniformly distributed within and around the mass. In malignant cases a feeding artery entering the tumor rather steeply at a right angle to the surface and traversing through the center was often noticed. The smaller blood vessels were rather randomly distributed over the volume with a preference to be more concentrated near the surface of the mass than within the center. In most cases the blood vessels were more easily seen in the color mode than in the power mode of Doppler imaging. This was a somewhat surprising result because the latter is relatively angle independent and thus should yield a more complete field of view. Figures 6-9 (attached at the end of the report) show some examples of color and power Doppler images of benign and malignant tumors. The blood vessels are shown in color. The left and right panels are the color and power Doppler images. The scale of mean velocity is shown on the right hand side of the color Doppler images as a palette bar. The top color of this bar represents $\pm$ 6.4 cm/sec. The color blue and red also seen in the blood vessels represent slow flow on the order of 1 cm/sec. The width of the blood vessels is on the order of 1 mm. This color Doppler images shown in Figure 6-8 have a close correspondence to the power Doppler images.

Figure 6 shows a case of fibroadenoma with mild vascularity. (This is typical for fibroadenoma). However, in some cases, especially in younger patients, high vascularity was noticed. This is shown in Figure 7. The pattern and magnitude of vascularity is very

similar to that noticed in the malignant tumors shown in Figures 8 and 9. This case represents an example where diagnosis on the basis of vascularity alone is confusing. However, when the B-mode imaging was considered, the gray scale information for this case allowed the diagnosis to be fibroadenoma. The color and power Doppler images of malignant tumors show a very distinct pattern of blood vessels. Two cases are shown in Figures 8 and 9. The blood vessels traverse through the core of the lesion. The weak scattered coloration seen in power Doppler images (Figure 8 lower right panel) is due to the "flash artifact" caused by tissue and/or transducer motion.

Quantitative measurements of vascularity within the boundaries of the detected mass

The quantitative measurements of vascularity are summarized in Table 2. The values reported in the table correspond to the tissue volume enclosed within the tumor boundaries. The standard deviation values given represent the spread over patient population studied. The magnitude of this spread (viz., standard deviation) is comparable to the mean values. The implication of this result is that the variation in vascularity from one patient to another is significant. However, when the data is pooled together there is a definite pattern. The mean vascularity by each of the six measures given in the table is higher for the malignant tumors as compared to the benign masses. Thus, although the diagnosis on the basis of mean values on a case by case basis may not be always feasible, the use of this technique to determine population trends may be useful.

	Power Doppler			Color Doppler		
	MCL	% Area	CWFA	MCL	% Area	CWFA
Scale ----->	(0-100)	(0-100)	(0-100)	(0-100)	(0-100)	(0-100)
Represents ->	Blood Vol. per Col. Pixel	Area of Perfusion	Blood Volume thru X-section	Mean Flow Vel. Col. Pixel	Area of Perfusion	Mean Blood flow thru X-section
Benign (B)	6.8 ± 5.5	8.9 ± 8.4	0.9 ± 0.9	27.2 ± 14.2	4.9 ± 5.2	2.2 ± 2.3
Malignant (M)	9.3 ± 5.2	12.8 ± 17.4	1.8 ± 2.8	34.7 ± 16.9	6.5 ± 4.5	3.8 ± 3.5
Ratio M/B	1.37	1.43	2	1.28	1.33	1.73
% Difference	37	43	100	28	33	73

Table 2: Mean values ± standard deviation of vascularity measured within the boundaries of the suspicious masses. MCL, percent area, and CFWA represent mean color level, area covered by the blood vessels, and color weighted area of perfusion, respectively.

The MCL, percentage area and CWFA values for larger blood vessels like the carotid artery using the imaging parameters used in this study approach a value of 100. The lower values for vascularity in Table 2 clearly demonstrate considerably less blood flow in the breast. This is in accordance with expectations because the breast in not a highly perfused organ. The MCL of power Doppler in the breast is 5 - 10 % of the blood in larger blood vessels (representative of systemic blood). Since each color pixel represents blood vessels, the lower value of MCL implies the blood volume in the breast vasculature has a lower proportion of red blood cells and a larger proportion of plasma than blood in the larger blood vessels. The lower local hematocrit in breast may explain why it was more difficult to image the blood vessels with power Doppler as compared to color Doppler, even though the former is angle independent and known for measuring slow flow. Color

Doppler imaging depends on frequency shift and is relatively unaffected by the relative number of moving red blood cells. The lower hematocrit in smaller vessels has been previously documented by several researchers using invasive and nuclear tracer studies. The microvascular hematocrit is an important determinant of blood viscosity and oxygen transport. These results show ultrasound provides a simple non-invasive method to determine local hematocrit in small vessels. Another possible explanation for lower MCL values could be that each pixel consists of several smaller vessels of the size of capillaries or larger. That is, each pixel is composed partly of blood and partly of tissue with no blood. Under this condition the lower MCL value is likely to be due to lesser blood volume. However, this explanation is unlikely because the current state of art imaging uses wall filters and signal processing schemes to minimize flash artifacts and does not detect flow through small vessels.

The percent area measurements show 5 to 15 % of the tumor cross-sections have blood flow. The color-Doppler values are lower primarily because it does not visualize blood vessels with flow orthogonal to the direction of the ultrasound beam. Due to slow flow velocity and low hematocrit the CWFA values range from 1 to 4 %.

The vessel count (number of vessels per mm^2) using two stains, F8 and CD31, is given in Table 3. There is a general agreement between the two stains. In most cases the F8 stain was stronger than the CD31 stain. With F8 it was easier to detect the blood vessels. The vessel count for malignant masses was greater than benign ones by an average of approximately 25%. This increase is comparable to the increase in percentage area noted with color and power Doppler imaging (see tables 2 and 3).

Table 3

Stain -->	F-8	CD-31
Benign (B)	54.6 ± 11.8	38.8 ± 12.5
Malignant (M)	64.2 ± 24.6	50.7 ± 21.8
Ratio M/B	1.18	1.31
% Difference	18	31

Quantitative measurements of regional vascularity

The data set of regional measurements is large and complex. To better appreciate the trends the data is displayed as color-coded bar diagrams in Figures 10-17. Each figure displays 8 measurements. The red and blue colors correspond to malignant and benign masses, respectively. The three bars in each color correspond to the shell surrounding the mass, peripheral shell within the mass, and the center of the mass, respectively. The yellow colored bars represent the mean of the three regions. From this data the following trends emerge.

(I) In all cases, without exception, the vascularity measures (MCL, percentage area, CWFA and histologic count) of the malignant tissues are greater than those for benign masses. Compare the blue and red bars in Figure 10-17.

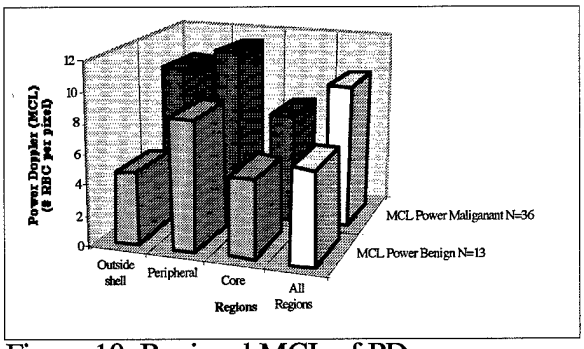


Figure 10: Regional MCL of PD

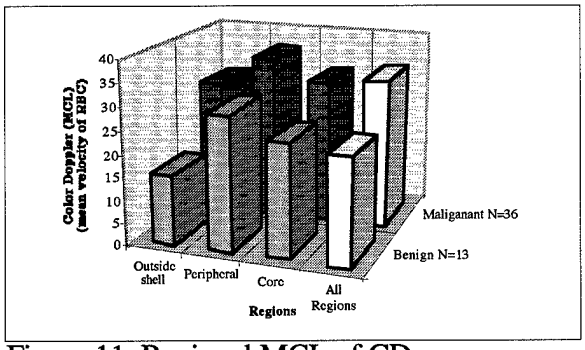


Figure 11: Regional MCL of CD

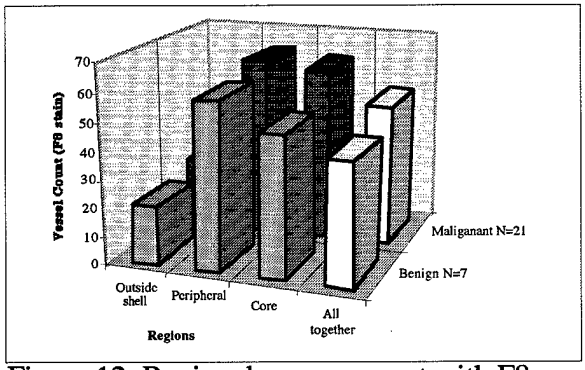


Figure 12: Regional measurement with F8

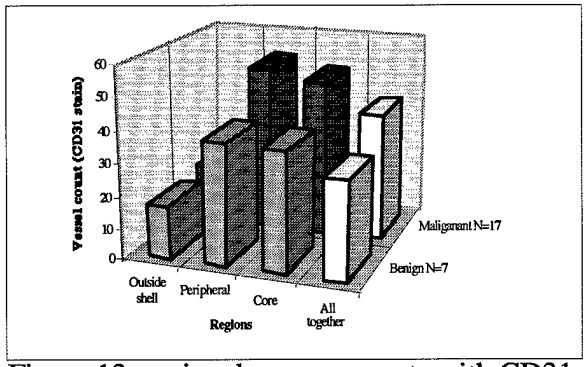


Figure 13: regional measurements with CD31

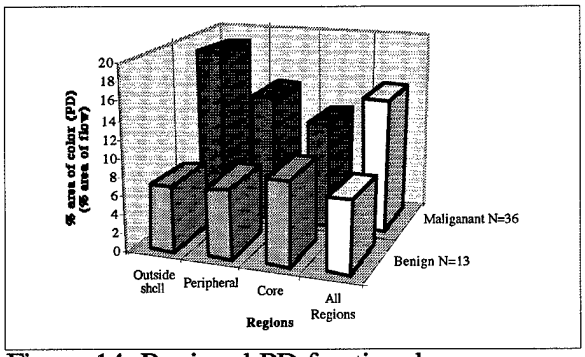


Figure 14: Regional PD fractional area

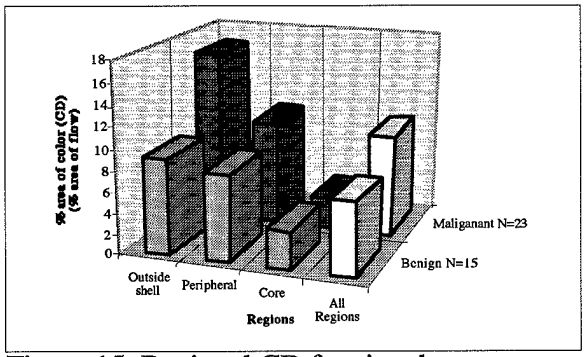


Figure 15: Regional CD fractional area

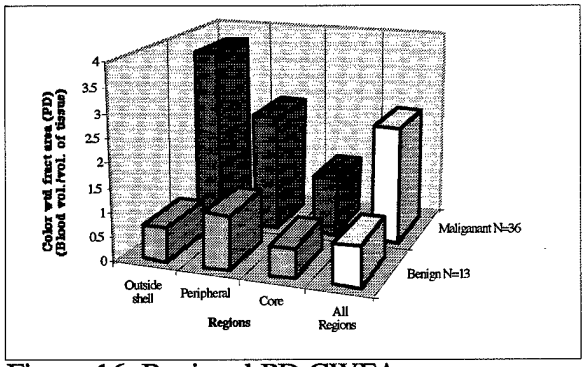


Figure 16: Regional PD CWFA

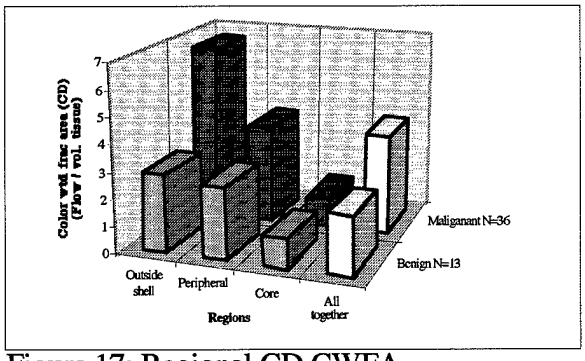


Figure 17: Regional CD CWFA

(II) The MCL of PD and CD images increase in the order: (outer shell) < (center - core) < (peripheral shell within the mass). See Figures 10 and 11. Both F8 and CD31 stains show vascularity to increase in the order (outer shell) < (center - core) < (peripheral shell within the mass). See Figures 12 and 13. The trend in MCL of PD and CD images correlate very closely with the histologic vessel count.

(III) The change in percentage area and CWFA follow a different pattern than the MCL. In malignant masses the percentage area and CWFM decreases uniformly as one travels from the outside shell to the center of the mass, i.e., in the order: center < peripheral shell < outside shell. See Figures 14 to 17. In contrast to the malignant masses the benign masses have a relatively uniform distribution of vascularity, i.e., center $\simeq$ peripheral shell $\simeq$ outside shell.

6.3. Discussion

In this study we have expanded our measurements to include regional variations in the breast vascularity. These measurements are an important addition and demonstrate how ultrasound imaging can be used to study tumor biology. The trends and the patterns in the vascularity of the entire lesion noted here are very similar to those reported in last years report. This gives us confidence that the conclusions drawn from this small study should serve as a good statistical sample.

Flow was detected in nearly all tumor studies. These results suggest that color and power Doppler imaging have sufficient sensitivity to detect blood flow through small blood vessels of 1 mm or less in diameter. The blood flow through these vessels is slow and on the order of 1 cm/sec. Both these results, viz., small vessels and slow flow, suggest that Doppler ultrasound enables visualization of blood vessels at the level of arterioles and venules.

On a case by case basis a considerable overlap was noted in the vascularity of the benign and malignant masses. The overlap was noted both in the qualitative and quantitative assessment of the images. Because in our study the imaging parameters were carefully controlled, and for the most part kept constant, we believe that the overlap is primarily due to the differences in blood flow from one patient to another. We did not account for potential cyclic variation in blood flow related to the menstrual cycle, or the menopausal status of a patient. Whether these factors played any role in contributing to the overlap between the two groups is not yet known. Nevertheless, this study shows that although measuring vascularity of the breast lesion increases the confidence of diagnosis, it cannot be used alone as an indicator to differentiate between the benign and malignant masses. However, when the data from all the patients are pooled together, clear and definite trends emerge in the mean values of the vascularity measurements.

The mean velocity of flow (MCL of Color Doppler) through malignant tumors is 1.3 times (30%) greater than that in the benign lesions (Table 2). The red blood cell density in malignant masses (MCL of power Doppler) is 1.37 times (37%) greater than the density in benign lesions. The diameter of microvessels is known to be larger in cancers than the diameter in normal tissues. Therefore, the cancerous tissues will have less pronounced Fahraeneus and Fahraeus-Lindquest effect. This means the reduction in the RBC density in the microvessels from the systemic value is less pronounced, and one should expect a RBC (density in tumors) to be higher than in normal tissues. This is precisely what the MCL measurements show.

The percentage area of the vascular compartment is larger for malignant masses by approximately 30-40 % (Table 2). In principle, this measure should correlate with the number of blood vessels. The histologic measurements based on the F8 stain show the

vessel count per unit area to be larger in malignant cancers as compared to the benign lesions. The ratio of vessel count for malignant and benign lesion is 1.25. This compares very favorably to the ratios of 1.33 -1.43 measured using Doppler imaging.

The mean blood flow for malignant cases is 1.73 time the flow in benign lesions. Very little information is available on blood flow and blood volume in human malignancy. According to one report the mean blood flow in post menopausal women is on the order of 0.04 ml/gm /min and it increases to 0.08 - 0.8 ml/gm /min in breast cancers [16] The increase in flow in cancers is about 2 to 20 times the normal flow. Our measurements are at the low end of the spectrum. However, they demonstrate an increase in accordance with the general expectation of increased blood flow in cancers [16]

The mean age of patients with benign masses is 44.5 ± 14 years, as compared to 59.4 ± 14 of the patient with malignant masses. Since the vascularity of the malignant masses is higher than that for the benign ones we considered the possibility that the measured difference in the vascularity was imply age related, and not a true measure of tumor biology. Figure shows a plot of vascularity against age of the patients. In this graph color weighted fractional area was used as a measure of vascularity. No correlation was observed ($R^2 = 0.0029$). Other measures of vascularity showed even less correlation. These results show that the measured difference is not due to the age difference of the two groups.

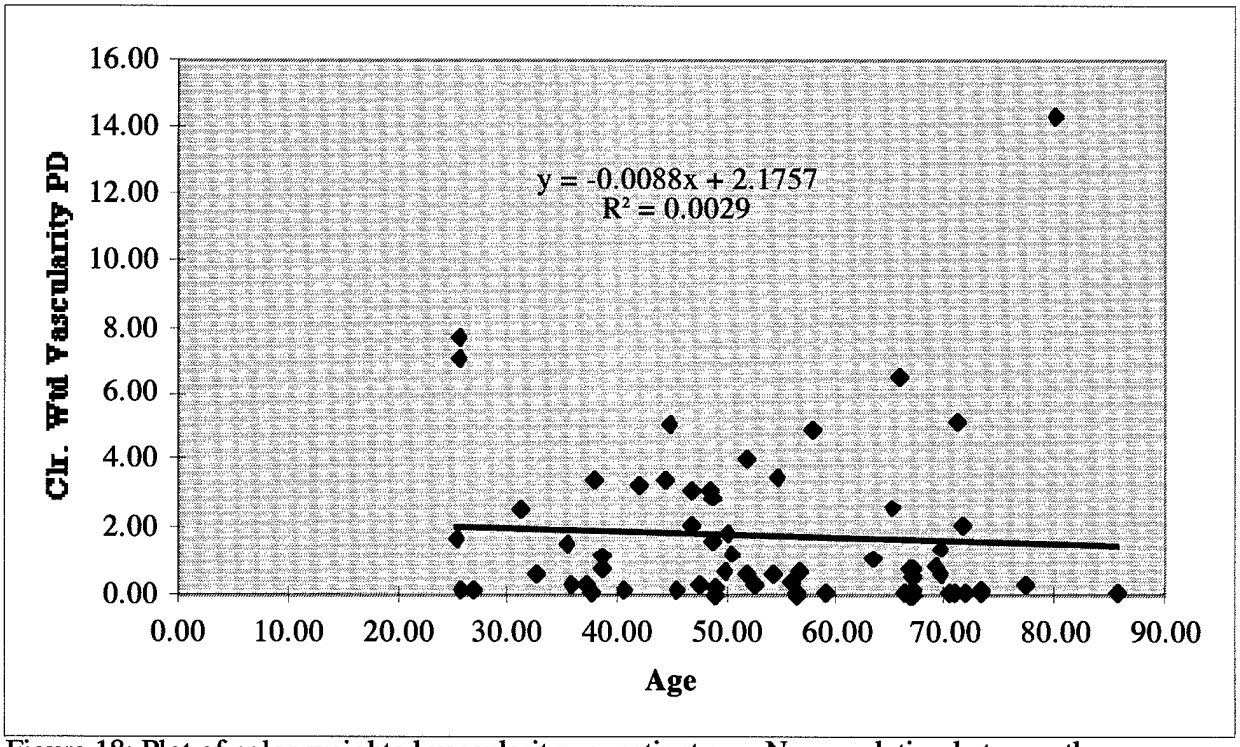


Figure 18: Plot of color-weighted vascularity vs. patient age. No correlation between the two parameters is observed (R^2= 00029).

The tumor vasculature is known to be highly heterogeneous and very different compared to normal vascular organization [17]. Based on our studies the gross architecture of tumor vasculature is characterized in three categories, the region around the tumor, the shell in the periphery but within the tumor mass, and the central region of the tumor. Our measurements of percentage area of perfusion show that the vascular compartment of malignant tumors decrease

progressively from outside the shell to the center. In contrast to this pattern the vascular compartment of the benign masses is relatively constant throughout the mass of the tumor. This pattern of regional variations in tumor vascularity fits well with the concepts of tumor biology. The neovascularization occurs particularly at the periphery [18]. The new vessels anastamoses with the host capillaries thus permitting an access of a wide network of blood vessels for tumor growth. With neoplastic growth the blood pressure in the new vessels increases 3-4 fold from 30-40 cm H_20 to 120-130 cm H_20. This pressure change deforms and dilates the post-capillaries and reduces the intracapillary pressure. Furthermore, it is known that the newly formed tumor blood vessels tend to be leaky and transport fluid from blood to the interstitium. This can increase the interstitial pressure by as much as 45 mm Hg. There is a wide body of literature on measuring these pressures inside the tumors. These studies demonstrate the interstitial pressure to be higher in the center of the cancer than at the boundary of the mass. The reduced intravascular pressure and the increased interstitial pressure means that the blood vessels in the center of the mass must either collapse, or, if they have to stay open the intravascular pressure must increase beyond 45 mm Hg. In either case, the blood flow to the center will be impeded and reduce thereby vascularity. All our measurements demonstrate this to be the case. Each measure of vascularity (viz. MCL, percent area and CWFA) is lower for the central region compared to the periphery of the cancer. The most exciting thing about these results is that these measurements can be made on patients with least intervention by using commercial ultrasound scanners and our data analysis software. Furthermore, it is quite reasonable to expect that the slopes or the gradients of blood volume and blood flow derived from ultrasound image measurements are indirect measures of pressure gradients in the tumor. These pressure gradients have a considerable clinical significance because they have been attributed to be related to the difficulties associated with drug delivery to the site of cancers. The regional measurements of vascularity could prove to be most valuable in assessing these difficulties.

Another way to view the regional vascularity gradient is as a measure of supply and demand equation for tumor masses. Uniform distribution of vascularity (near zero slope) observed for the benign lesions imply a balance between the supply and demand of blood to the lesion. A gradient in vascularity, on the other hand, is representative of a mismatch between the blood supply available at the periphery of the mass and the amount that gets to the center. For example, a steep gradient would imply that only a small fraction of the available blood supply is likely to reach the center of the mass. This in turn would indicate a greater possibility of tumor necrosis.

Although it is early, our results on regional measurements are very promising and they open new exciting possibilities of studying tumor biology noninvasively in the patient population.

7. Conclusions

This study demonstrates the potential and the limitations of Doppler imaging in measuring vascularity to assess breast cancer. Specifically it provides insight into the following questions.

Is Doppler imaging a versatile tool to image vascularity of the breast? Blood vessels were seen in all case except one. The imaging was easy to perform on a routine basis and the masses detected by mammography were seen in all cases except two. A successful completion of this study demonstrates ultrasound to be a versatile, noninvasive tool to evaluate the vascularity of the breast lesions.

Is vascularity a good measure to evaluate breast tumors? The results on this issue from this study are mixed. On a case by case basis there is a notable overlap between the values of vascularity of the malignant and benign lesions. Interestingly, the histologic

measurements considered to be the gold standard has a comparable overlap in the vascularity values. When the vascularity was used as one of the parameter in conjunction with B-mode imaging it increased the confidence level of diagnosis.

The mean values of vascularity measurements from ultrasound imaging follow a well define pattern consistent with the previous knowledge of tumor blood flow. By all our measures malignant breast cancers were on average more vascular than the benign masses. These results are of particular interest as they demonstrate the utility of using ultrasound Doppler imaging for conducting population studies.

What is the cause of overlap in the vascularity values? In view of the robustness of Doppler imaging and the strict protocol we followed in controlling the imaging parameters we believe most overlap is due to the intrinsic variation in blood flow from one patient to another. A confounding factor that can potentially change this view is if there is a significant variation in blood flow throughout the menstrual cycle.

Do ultrasound and histologic measurements agree with one another? The two measurements are different aspects of vascularity. Both modes show cancers to be more vascular and are thus in agreement with one another.

Can Doppler imaging measure heterogeneity in tumor vasculature? The results of this study clearly indicate that this can be easily achieved. The measures derived based on ultrasound imaging are consistent with our prior knowledge of tumor biology and blood flow. The attractive aspect of these results is that the measurements are made noninvasively in patients and can be repeatedly made as a function of time. We are particularly excited with these results as they provide new possibilities in evaluating local changes in hematocrit and the associated oxygen transport, in assessing supply-demand equation of tumors and its relationship to the rate of growth or control, and even the possibility of indirectly assessing interstitial pressures. Although each of these aspects have yet to be realized they offer promise for objective evaluation of patients with breast cancer. These results will be presented at the 1998 meeting of the Radiological Society of North America. We hope to finally write these results for publication in a peer reviewed journal.

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Benign
(fibroadenoma)

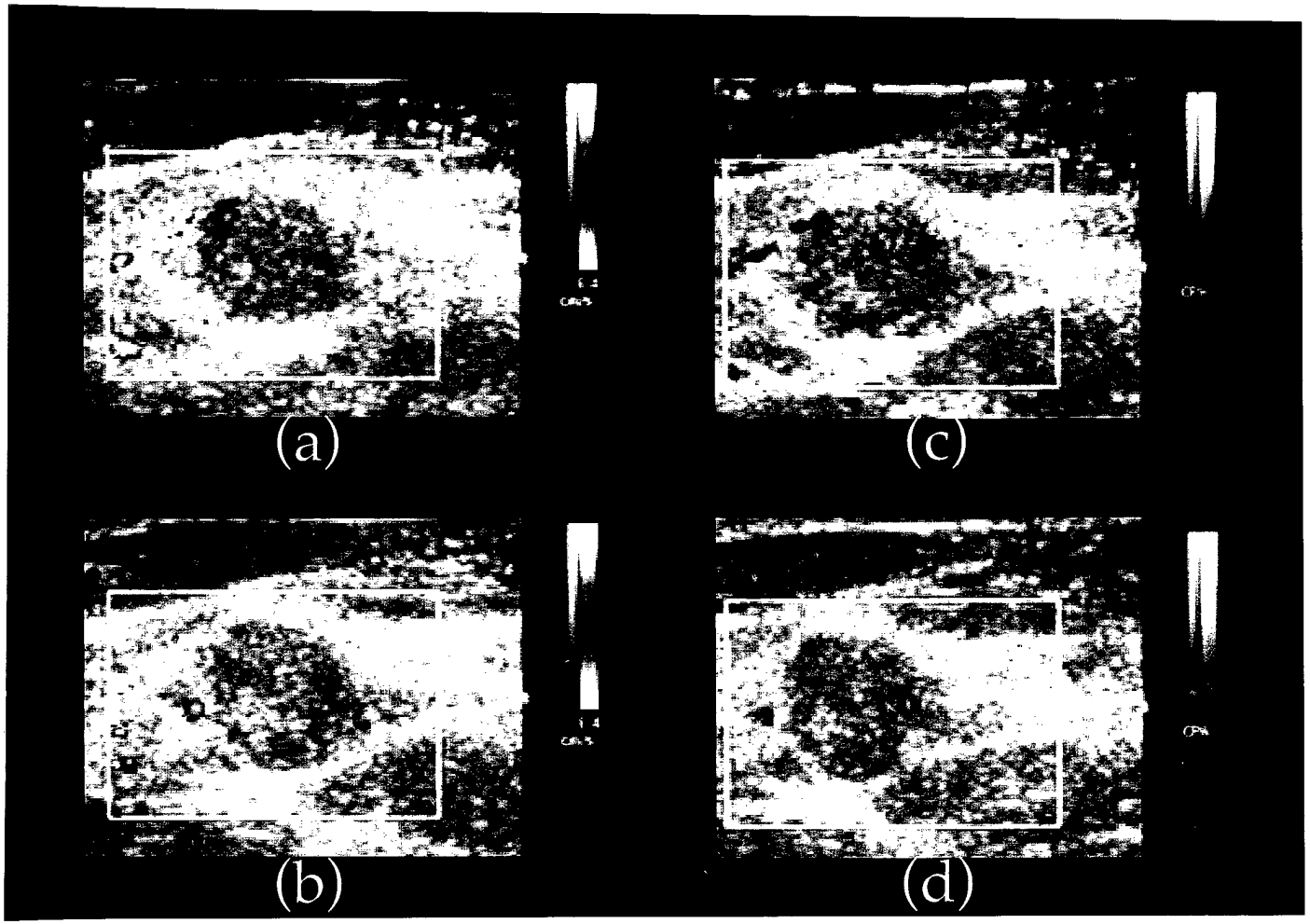

Figure 6

Benign
(fibroadenoma)

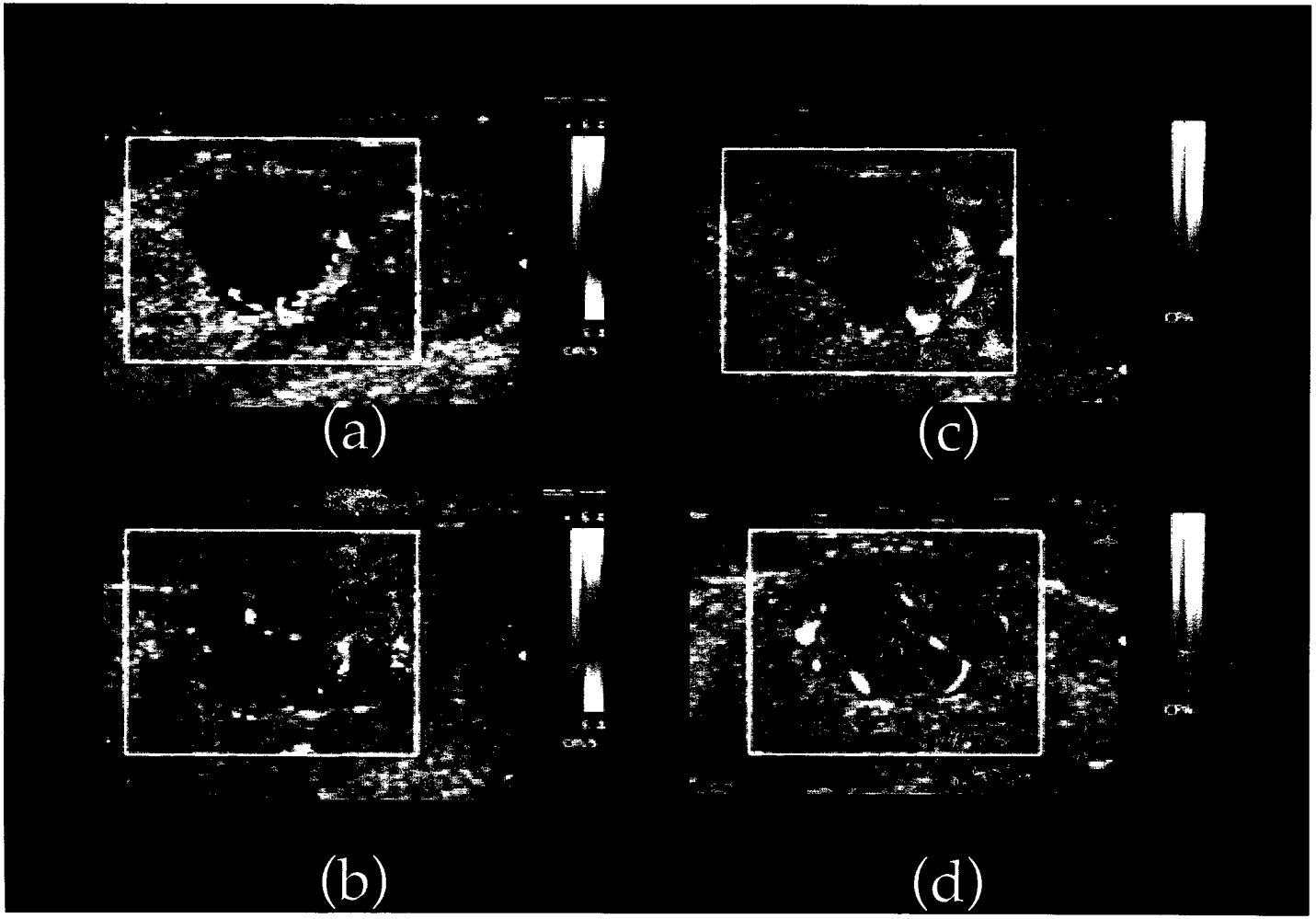

Figure 7

Malignant
(Inf. Ductal CA)

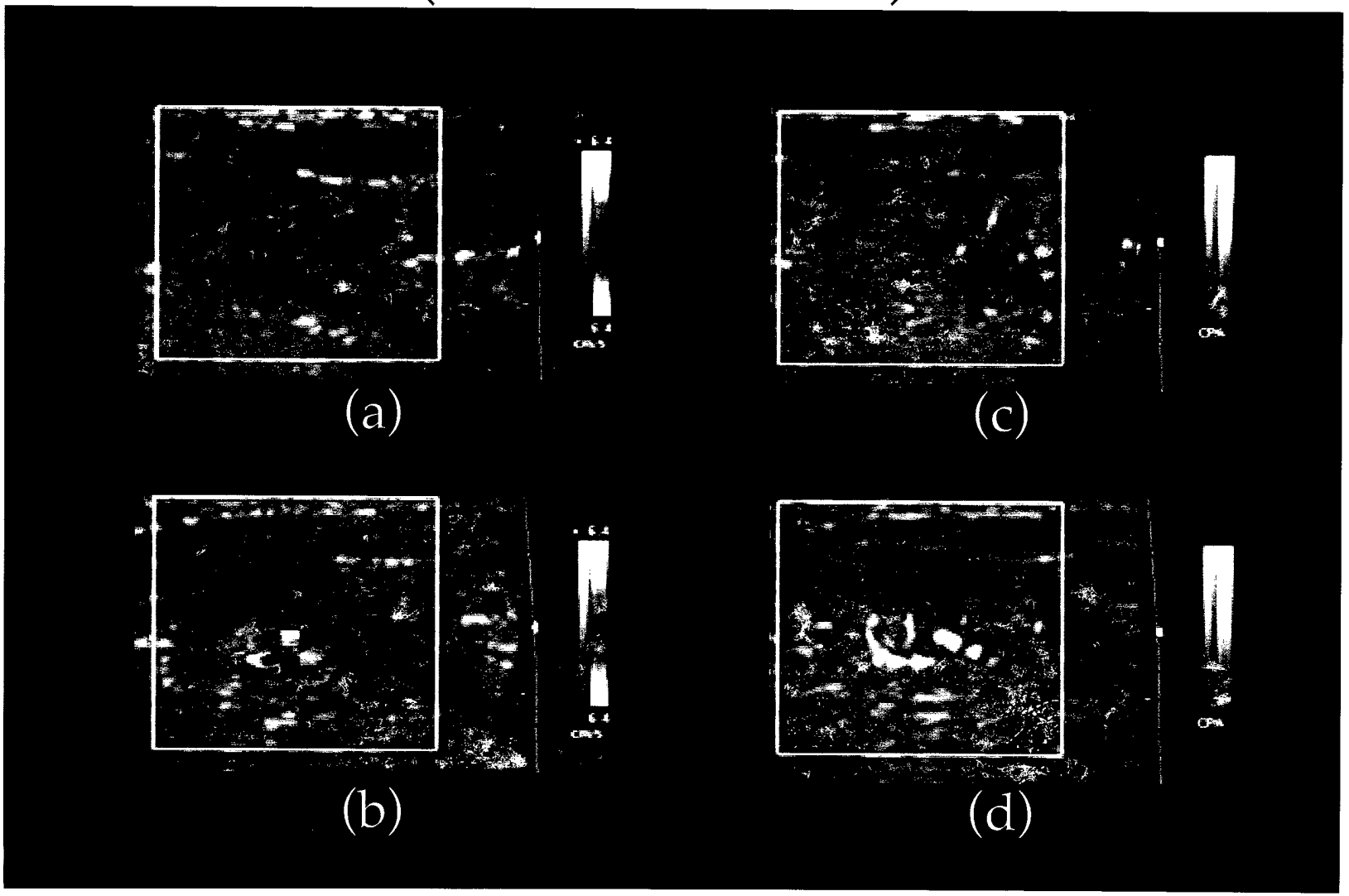

Figure 8

Malignant
(metaplastic carcinoma)

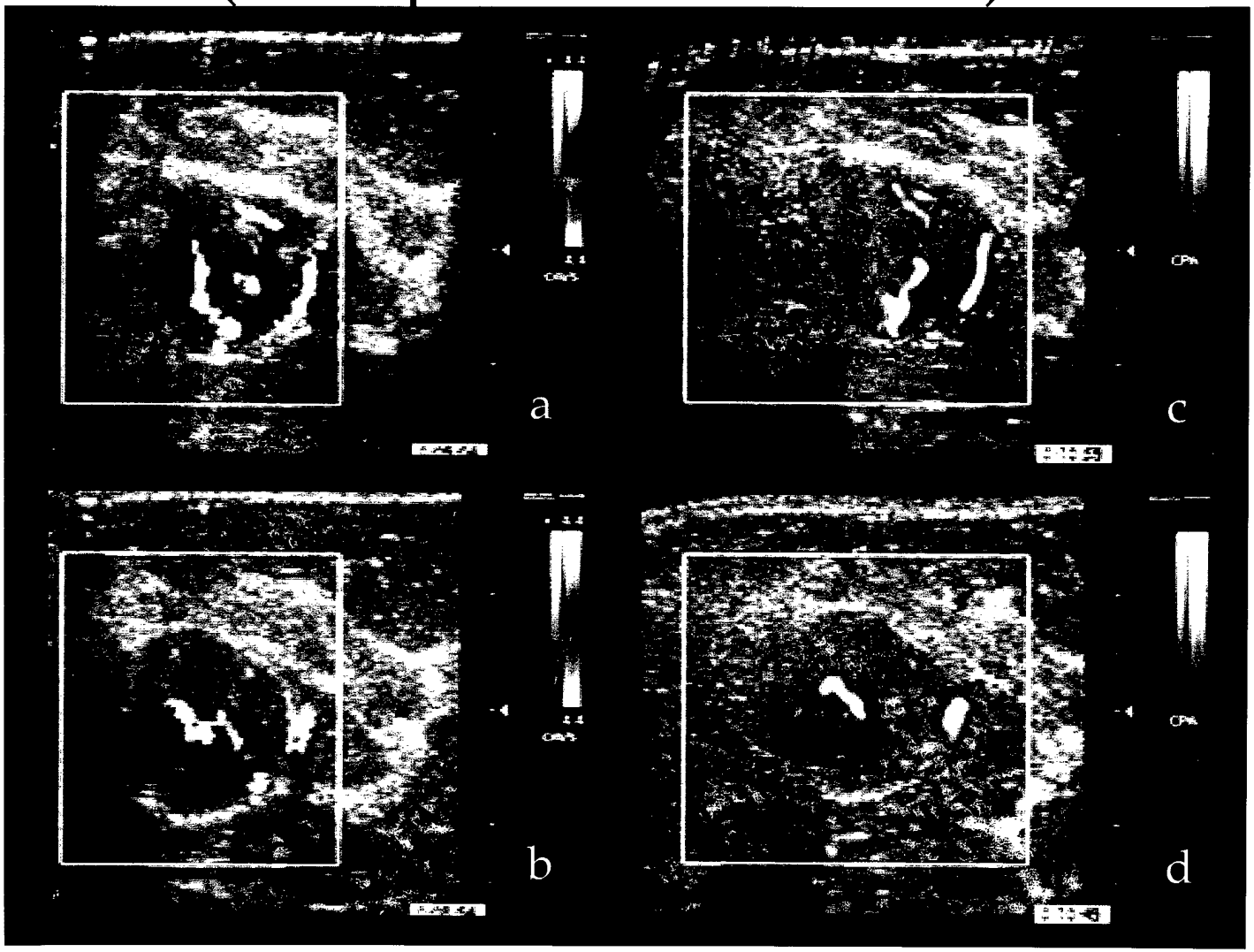

Figure 9